

不同钙制剂对‘寒富’苹果果实硬度及 相关细胞壁代谢物质的影响

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摘要:【目的】探讨不同类型钙制剂对果实硬度影响的生理机制,为有效延长果实货架期、维持果实品质提供理论依据和技术支撑。【方法】以硝酸钙、氯化钙、氨基酸钙、糖醇钙为钙制剂,对‘寒富’苹果进行采后浸钙处理,在货架期内动态监测果实硬度、细胞壁物质组分、细胞壁降解酶活性等指标。【结果】采后‘寒富’苹果果实硬度、原果胶含量、纤维素含量呈下降趋势,而果实可溶性果胶、果胶甲酯酶(PME)、多聚半乳糖醛酸酶(PG)、纤维素酶(CX)酶活性均呈上升趋势。4种形态钙制剂处理均提高了果实硬度,抑制了果实原果胶和纤维素下降以及可溶性果胶上升,抑制了PG、CX和PME酶活性。至贮藏35 d时,硝酸钙、氯化钙、氨基酸钙、糖醇钙处理的果实与对照相比,硬度分别增加了4.0%、6.0%、6.6%和7.9%;原果胶含量比对照高17.2%、21.0%、25.3%和29.6%;纤维素含量比对照高28.9%、36.5%、53.3%和68.5%;可溶性果胶比对照分别低13.7%、5.0%、19.4%和26.6%;PME活性比对照果实分别低19.8%、21.9%、27.9%、31.8%;PG酶活性比对照果实分别低5.4%、8.7%、15.3%、19.6%;CX酶活性比对照果实酶活性分别低22.1%、22.6%、43.7%、50.1%。相关性结果显示:果实硬度与可溶性果胶($p < 0.01$)、PG酶($p < 0.01$)、CX酶($p < 0.01$)、PME酶($p < 0.05$)均呈显著负相关,而与原果胶、纤维素呈显著正相关($p < 0.05$)。【结论】采后钙处理能显著降低‘寒富’苹果PME、PG、CX酶活性,抑制原果胶和纤维素的降解和可溶性果胶的上升,从而更好地维持果实硬度。4种钙制剂中以糖醇钙处理效果最优。

关键词:苹果; 钙处理; 硬度; 细胞壁

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Effects of different calcium agents on fruit firmness and related cell wall metabolites in ‘Hanfu’ apple

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Abstract:【Objectives】After different calcium treatments, fruit firmness, cell wall components and enzyme activities of apple fruit were measured to explore the physiological mechanism of the effects of treatments with different types of calcium on the fruit firmness and to provide a reference to extending shelf life and maintaining fruit quality.【Methods】Firm ripe, medium and uniform sized ‘Hanfu’ apple were hand-harvested with stalk in the morning from an orchard in Xingcheng, Liaoning, China, and were transported to the laboratory in the day. A total of 400 fruit were randomly divided into five groups and 10 fruit were randomly selected to determine the initial values of various parameters, and the remaining fruit were used for calcium treatments. Fruit of the five groups were immersed in calcium nitrate, calcium chloride, calcium amino acid, calcium sorbitol, or in clear water as control group. The

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concentration of calcium ion in each treatment was 2% and immersion time was 15 min. The fruit were then stored at room temperature. For each group, 10 fruit were randomly sampled every 7 d during storage. The fruit firmness, soluble pectin, protopectin, cellulose, and the activities of pectin methylesterase (PME), polygalacturonase (PG) and cellulase (CX) were monitored during storage to explore the relationship between fruit firmness and the metabolites of cell walls. 【Results】Fruit firmness is the basis of fruit storage. With the extension of storage, fruit firmness decreased rapidly for the first 7 days and decreased slowly from day 7 to day 28. The four calcium treatments all increased the fruit firmness. At 35 days of storage, the firmness of fruit treated with calcium sorbitol was significantly higher than that of the control, while the other Ca treatments were not significantly different from the control. Compared to the control, the firmness of fruit treated by calcium nitrate, calcium chloride, calcium amino acid and calcium sorbitol was increased 4%, 6%, 6.6% and 7.9%, respectively. During storage, the content of soluble pectin increased rapidly for the first 7 days and increased steadily from day 7 to day 35. The content of the soluble pectin of all the calcium treatments was lower than that of the control, indicating that calcium treatments could significantly inhibit the increase of soluble pectin content in fruit. At 35 days of storage, the content of soluble pectin treated with calcium amino acid, calcium sorbitol or calcium nitrate was significantly lower than that of the control, while there was no significant difference between calcium chloride treatment and the control. The content of soluble pectin in the treatments of calcium nitrate, calcium chloride, calcium amino acid and calcium sorbitol was 13.7%, 5.0%, 19.4% and 26.6% lower than in the control, respectively. The contents of the protopectin and cellulose decreased during storage and were higher in all calcium treatments than in the control, indicating that calcium treatments could effectively inhibit the decline of protopectin and cellulose in fruits. At 35 days of storage, the content of protopectin was 17.2%, 21.0%, 25.3% and 29.6% higher and the content of cellulose 28.9%, 36.5%, 53.3% and 68.5% higher than that of the control in the treatments of calcium nitrate, calcium chloride, calcium amino acid and calcium sorbitol, respectively. Cell wall degrading enzymes play an important role in fruit softening. During the storage, the activities of PME, PG and CX all increased and calcium treatments inhibited the increase in the enzyme activities, as they were all significantly lower compared with the control. At 35 days of storage, the activity of PME was 19.8%, 21.9%, 27.9% and 31.8% lower; the activity of PG 5.4%, 8.7%, 15.3%, 19.6% lower, and the activity of CX 22.1%, 22.6%, 43.7%, 50.1% lower than the control in the treatments with calcium nitrate, calcium chloride, calcium amino acid and calcium sorbitol, respectively. Correlation analysis showed that fruit firmness was significantly negatively correlated with soluble pectin, and PG, CX and PME activities, but significantly positively correlated with protopectin and cellulose. 【Conclusion】Postharvest calcium treatments could significantly reduce the activities of PME, PG and CX enzymes, inhibit the degradation of protopectin and cellulose, and suppress the rise of soluble pectin, and thus maintain the fruit firmness. Based on our results, application of calcium sorbitol gave the best effect among all the treatments, and therefore it is recommended for commercial use.

Key words: Apple; Calcium treatment; Firmness; Cell wall

截至 2016 年,全国‘寒富’苹果栽培面积超过 14 万 hm²,辽宁省‘寒富’苹果栽培面积达到 12 万 hm²,占全省苹果栽培面积的 45.6%,成为辽宁省苹果栽培的第一大品种以及全国栽培面积最大的自育品种^[1],并且栽培面积还在不断的增加。由于‘寒富’苹果产量高、果实较大,生产中常会出现果实钙

素失调而诱发的苦痘病、水心病、裂果等生理病害,严重降低果实的商品价值,给果农带来很大的经济损失。钙是细胞壁的重要组成成分,具有维持细胞壁和细胞膜稳定的功能^[2]。研究表明,外源钙处理能有效抑制果实硬度的下降^[3],降低细胞壁降解酶的活性^[4],维持细胞壁结构的稳定,提高植物的抗逆

性、抗病性,减少生理病害的发生,延长贮藏期,促进果品质的提高^[5]。目前,国内外学者关于钙处理对采后果实生理影响的报道较多。如采后对中华猕猴桃进行CaCl₂处理,可以降低纤维素酶活性,延缓果实硬度下降,延缓果实软化衰老进程^[6];采后对‘富士’苹果进行CaCl₂处理能有效保持苹果的硬度和减缓可溶性果胶的增加,延缓果实的软化进程^[7];采后对‘金冠’苹果进行CaCl₂处理提高了果实硬度,减少了苦痘病的发生^[8];采后对‘黄花’梨进行CaCl₂浸泡处理明显抑制了果胶的降解与PG的活力,提高了果实硬度^[9];采后对葡萄柚进行CaCl₂处理降低了果实可溶性果胶含量,有效控制了果实硬度下降^[10];采后对杏进行浸钙处理,可以抑制细胞壁多糖物质的降解,维持果实的硬度^[11]。但目前对‘寒富’苹果采后钙处理研究较少且多集中在单一类型的钙制剂,以CaCl₂处理较多,对采后不同钙制剂处理之间的区别研究较少。

笔者以‘寒富’苹果为试材,采用实验室自制不同形态钙制剂对采后‘寒富’苹果进行处理,研究不同类型钙制剂对苹果果实硬度、细胞壁组分及细胞壁降解酶活性变化的影响,以期获得能有效延长果实货架期的最佳钙制剂,并阐明该种形态钙制剂影响果实硬度的生理机制,为果品质维持提供理论和技术依据。

1 材料和方法

1.1 供试材料与方法

试验于2017年10月至12月在中国农业科学院果树研究所进行,供试品种为‘寒富’苹果,树龄10 a(年)。于2017年10月18日上午采收,取样时分别从树冠中部外围,随机采取果形端正、大小均匀、无病虫害的果实共400个。采收后当天运回实验室于室温放置作为试验材料。

供试钙制剂:(1)硝酸钙[Ca(NO₃)₂·4H₂O,分析纯];(2)氯化钙[CaCl₂,分析纯];(3)氨基酸钙(钙盐与有机小分子氨基酸螯合,实验室自制);(4)糖醇钙(钙盐与有机小分子糖醇螯合,实验室自制)。

样品处理:随机抽取10个苹果用作测定初始值,剩余苹果用于浸钙处理。

试验共设5个处理:(1)对照清水浸泡;(2)硝酸钙浸泡;(3)氯化钙浸泡;(4)氨基酸钙浸泡;(5)糖醇钙浸泡。不同钙制剂间保证钙离子浓度一致(Ca²⁺

质量分数为2%)。每个处理选取果实70个,在预先准备好的钙制剂中浸泡15 min,期间翻动苹果,保证样品浸泡程度一致。浸泡结束后,自然晾干,分装于果箱中,室温存放,每隔7 d各处理随机选取10个果实用于相关指标的测定,3次重复,共取样6次。

1.2 测定项目与方法

1.2.1 果实硬度测定 果实硬度采用物性分析仪测定,探头直径5 mm。随机取5个果实,在每个果实赤道面对称两侧测定,取平均值。

1.2.2 果实细胞壁物质的提取及含量测定 可溶性果胶与原果胶的提取及测定参考曹建康等^[12]的方法。纤维素含量的测定参考王学奎^[13]的方法。

1.2.3 细胞壁降解酶的提取及活性测定 酶液提取参照曹建康等^[11]的方法。取果肉1.0 g,放于预冷的研钵中加入5 mL预冷的95%(φ,后同)乙醇,冰浴条件下研磨成匀浆,全部转入到离心管中。4 ℃放置10 min后,于4 ℃,12 000 r·min⁻¹离心20 min。倾去上清液,向沉淀物中加入预冷的80%乙醇5 mL,4 ℃放置10 min后,相同方法离心。再倾去上清液,向沉淀物中加入8 mL提取液,4 ℃放置提取20 min,相同方法离心,收集上清液即为酶提取液,4 ℃保存。

果胶甲酯酶(PE)活性采用NaOH滴定法测定,以37 ℃条件下,每min每g鲜样催化果胶释放1 mmol的CH₃O⁻为一个酶活力单位。多聚半乳糖醛酸酶(PG)和纤维素酶(CX)活性均采用DNS比色法测定,分别以37 ℃条件下,每min每g鲜样分解果胶产生1 μg游离的半乳糖醛酸和分解羧甲基纤维素钠产生1 μg的葡萄糖为1个酶活力单位。以上各种酶活性测定均3次重复,取平均值。

1.3 数据处理

使用Microsoft Excel 2010软件对所获数据处理并绘图;使用SPSS19.0软件进行相关性分析和差异显著性分析。

2 结果与分析

2.1 不同钙处理对果实硬度的影响

从图1可以看出,随着货架期的延长,各处理之间果实硬度均呈下降趋势,在前7 d果实硬度迅速下降,7~28 d果实硬度下降缓慢,果实软化进程减慢。在存放过程中,各处理的果实硬度均高于对照,但各处理之间果实硬度变化差异不显著。至试验结束时

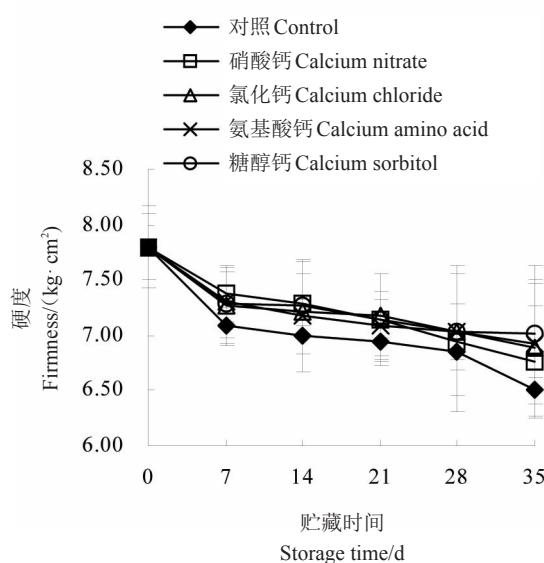


图 1 采后钙处理对果实硬度的影响

Fig. 1 Effect of different calcium treatments on firmness of apple fruit

(35 d), 糖醇钙处理的果实硬度显著高于对照($p < 0.05$), 较处理前下降了10.1%; 硝酸钙、氯化钙、氨基酸钙处理的果实硬度较处理前分别下降了13.3%、11.7%、11.2%, 而对照果实硬度下降了16.7%。说明采后钙处理可以有效地保持果实硬度, 延缓果实衰老, 且以糖醇钙处理果实硬度保持最好。

2.2 不同钙处理对可溶性果胶含量的影响

由图2可以看出, 随着货架期的延长, 果实的可溶性果胶含量呈持续上升趋势。各处理果实可溶性

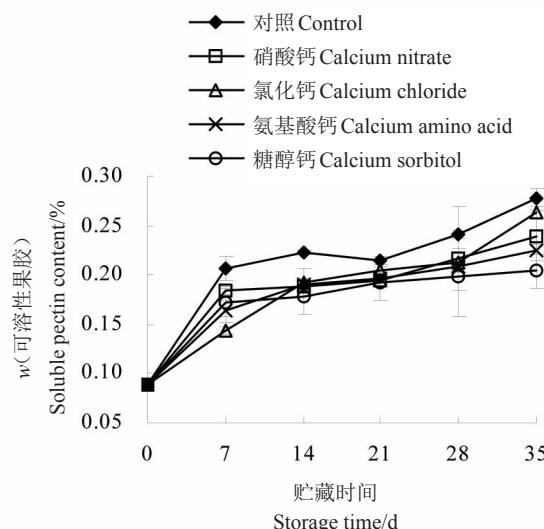


图 2 采后钙处理对果实可溶性果胶含量的影响

Fig. 2 Effect of postharvest calcium treatments on the changes in soluble pectin

果胶含量在前7 d迅速上升, 在7~35 d稳定上升。在存放过程中, 各处理果实可溶性果胶含量均低于对照。在7、14 d时, 各处理果实可溶性果胶含量均极显著低于对照果实($p < 0.01$); 在第35天时, 氨基酸钙和糖醇钙处理的果实可溶性果胶含量极显著低于对照果实, 硝酸钙处理的果实显著低于对照果实($p < 0.05$), 而氯化钙处理的果实与对照果实无显著差异。至35 d时, 4种钙制剂中, 糖醇钙处理的果实可溶性果胶含量比对照果实低26.6%; 硝酸钙、氯化钙、氨基酸钙处理的果实可溶性果胶含量比对照果实分别低13.6%、5.0%、19.4%。说明, 钙处理可显著抑制果实可溶性果胶含量的上升, 且糖醇钙处理能更好地抑制可溶性果胶的上升。

2.3 不同钙处理对原果胶含量的影响

由图3可以看出, 在货架期中, 果实原果胶含量与可溶性果胶含量变化趋势相反, 随着货架期时间的延长呈现下降趋势。同时可以看出, 浸钙处理的果实原果胶含量均高于对照果实。在28、35 d时, 糖醇钙处理的果实原果胶含量显著高于对照($p < 0.05$), 而其他钙处理果实与对照差异不明显。至35 d时, 4种钙制剂中, 糖醇钙处理的果实原果胶含量比对照果实高29.6%; 硝酸钙、氯化钙、氨基酸钙处理的果实原果胶含量比对照果实分别高17.2%、21.0%、25.3%。说明钙处理可有效抑制果实原果胶含量的下降, 且糖醇钙处理能更好地抑制果实原果胶含量的下降。

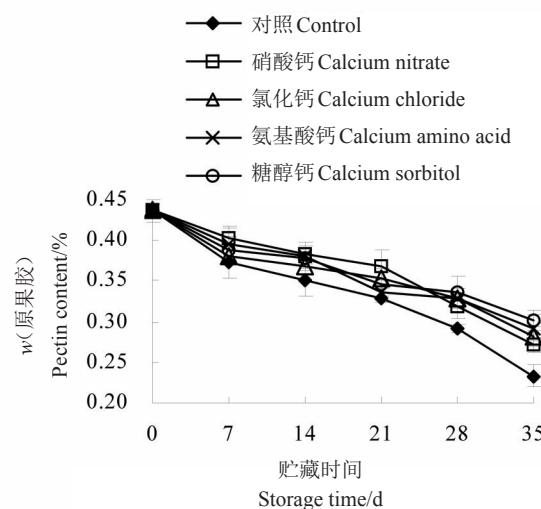


图 3 采后钙处理对果实原果胶含量的影响

Fig. 3 Effect of postharvest calcium treatments on the changes in protopectin

2.4 不同钙处理对纤维素含量的影响

由图4可知,果实纤维素含量与原果胶含量变化趋势相似,随着贮藏时间的延长呈下降趋势。各处理果实纤维素含量均高于对照。至28 d时,氨基酸钙和糖醇钙处理的果实纤维素含量显著高于对照果实($p < 0.05$),而氯化钙和硝酸钙处理的果实与对照无显著差异。至35 d时,4种钙制剂中,糖醇钙处理果实纤维素含量比对照果实高68.5%;硝酸钙、氯化钙、氨基酸钙处理果实纤维素含量比对照果实分别高28.9%、36.5%、53.3%。说明钙处理可有效抑制果实纤维素含量的下降,且糖醇钙处理抑制效果最好。

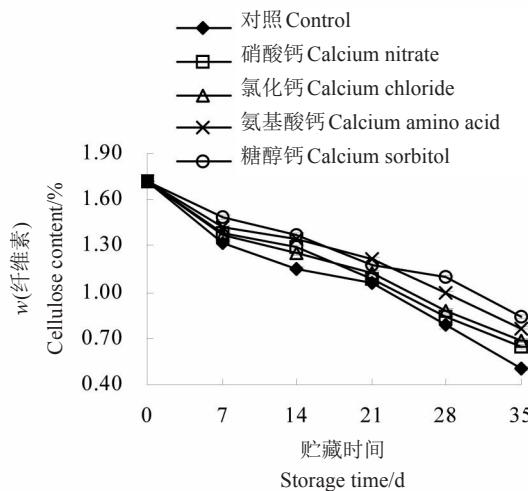


图4 采后钙处理对果实纤维素含量的影响

Fig. 4 Effect of postharvest calcium treatments on the changes in cellulose

2.5 不同钙处理对PME酶活性的影响

由图5可以看出,果实PME酶活性在放置前14 d呈现上升趋势,之后保持相对平稳水平甚至略有下降。在整个货架期过程中,各处理果实PME酶活性均极显著低于对照果实($p < 0.01$),且各处理之间也存在着显著差异($p < 0.05$)。至35 d时,糖醇钙处理的果实PME酶活性比对照果实低31.8%;硝酸钙、氯化钙、氨基酸钙处理的果实PME酶活性比对照果实分别低19.8%、21.9%、27.9%。说明钙处理可有效抑制果实PME酶活性的上升,且糖醇钙处理能更好地抑制PME酶的活性。

2.6 不同钙处理对PG酶活性的影响

图6表明,果实PG酶活性在货架期过程中整体呈现上升的趋势。同时,各处理果实PG酶活性均低于对照果实。经氯化钙、氨基酸钙、糖醇钙处理的果

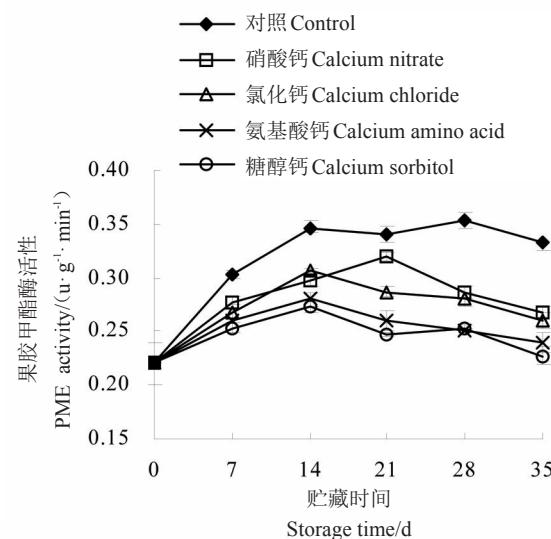


图5 采后钙处理对果实PME酶活性的影响

Fig. 5 Effect of postharvest calcium treatments on PME activity

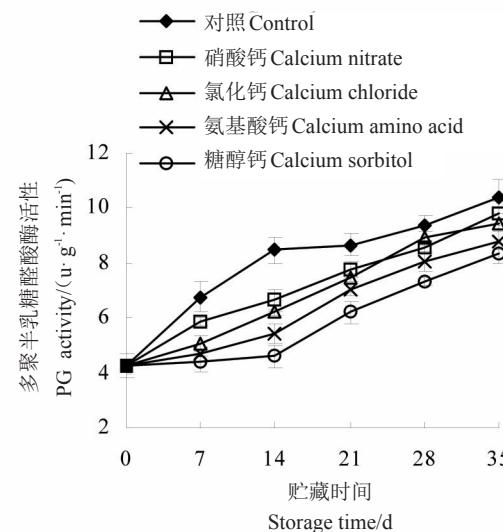


图6 采后钙处理对果实PG酶活性的影响

Fig. 6 Effect of postharvest calcium treatments on PG activity

实PG酶活性极显著低于对照果实($p < 0.01$),硝酸钙处理果实酶活性显著低于对照果实($p < 0.05$)。至35 d时,糖醇钙处理的果实PG酶活性比对照果实低19.6%;硝酸钙、氯化钙、氨基酸钙处理的果实PG活性比对照果实分别低5.4%、8.7%、15.3%。说明钙处理可有效抑制PG酶活性的上升。

2.7 不同钙处理对CX酶活性的影响

图7是果实在货架期CX酶活性变化的趋势。从中可以看出,各处理之间果实酶活性差异明显。对照果实整体呈明显上升趋势,经硝酸钙和氯化钙

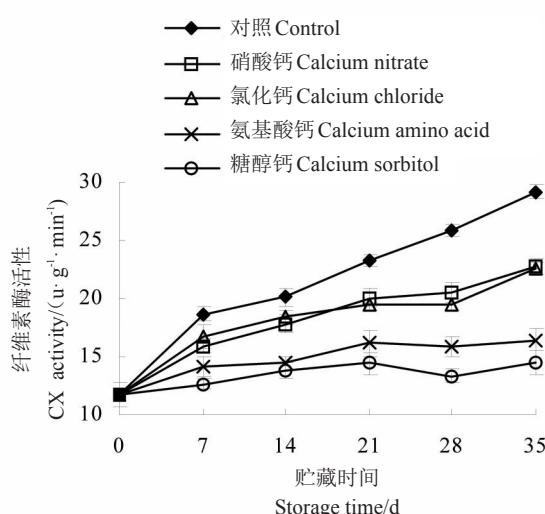


图 7 采后钙处理对果实 CX 酶活性的影响
Fig. 7 Effect of postharvest calcium treatments on CX activity

处理的果实在前 7 d 上升迅速,而后略有上升,而经氨基酸钙和糖醇钙处理的果实 CX 酶活性在整个货架期间一直保持相对平稳水平。各处理果实 CX 酶活性显著低于对照果实,且各处理之间也存在显著差异($p < 0.05$)。至 35 d 时,糖醇钙处理的果实 CX 酶活性比对照果实酶活性低 50.1%;硝酸钙、氯化钙、氨基酸钙处理的果实酶活性比对照果实酶活性分别低 22.1%、22.6%、43.7%。说明浸钙处理可显著抑制果实 CX 酶活性的上升,且糖醇钙处理抑制效果最好。

2.8 果实硬度、细胞壁组分及细胞壁降解酶相关性分析

由表 1 可以看出,‘寒富’苹果果实硬度与细胞壁组分及细胞壁降解酶之间存在着紧密的相关性。果实硬度与可溶性果胶、PG 以及 CX 呈极显著负相关,与原果胶、纤维素呈极显著正相关,而与 PME 酶呈显著负相关。同时,CX 和 PG 与可溶性果胶、原果胶、纤维素均呈极显著相关性,而 PME 与可溶性果

表 1 果实硬度、细胞壁组分及细胞壁降解酶的相关性
Table 1 The correlations among fruit firmness, cell wall compositions and their metabolizing enzyme activities

	硬度 Firmness	可溶性果胶 Soluble pectin	原果胶 Proto-pectin	纤维素 Cellulose
果胶甲酯酶 PME	-0.583*	0.595*	-0.646*	-0.661*
多聚半乳糖醛酸酶 PG	-0.777**	0.948**	-0.905**	-0.918**
纤维素酶 CX	-0.792**	0.938**	-0.954**	-0.965**
硬度 Firmness	1	-0.734**	0.843**	0.819**

胶、原果胶、纤维素呈显著相关性。说明细胞壁降解酶在果实软化进程中起着重要的作用。

3 讨 论

果实细胞壁物质主要由果胶、纤维素、半纤维素等多糖类物质组成,它们通过相互交联形成网状结构,使果实具有一定的形状和弹性,是果实硬度表现的物质基础^[14]。细胞壁结构和成分的改变是引起果实质地品质变化的主要原因^[15-16]。在果实衰老过程中,往往伴随着 PME 酶、PG 酶、CX 酶等细胞壁相关代谢酶活性的升高,导致原果胶、纤维素等细胞壁物质的分解,以及可溶性果胶含量的上升,最终导致果实硬度下降,质地变软^[17-18]。笔者也得到类似结果,果实硬度与细胞壁组分及细胞壁降解酶之间存在着显著相关性,‘寒富’苹果在贮藏过程中 PME 酶、PG 酶、CX 酶等细胞壁相关代谢酶活性逐渐升高,原果胶、纤维素含量下降,可溶性果胶含量上升,导致果实硬度下降,质地变软。

钙是构成细胞壁的重要元素,钙离子可与细胞壁中的果胶结合形成 Ca-果胶交联聚合物,这种聚合物能够增加细胞壁的机械强度,抑制中胶层的降解和细胞壁酶的降解作用,从而维持细胞壁结构的稳定性,进而使果实保持较好的质地品质^[19]。大量研究表明外源钙处理可以抑制原果胶、纤维素的降解,从而延缓果实硬度下降,延长果实贮藏期^[20-22]。本试验中,采用不同形态钙对‘寒富’苹果进行浸钙处理,均显著抑制了原果胶和纤维素的降解,可溶性果胶的增加,使果实在贮藏后期保持较高的硬度,这与前人的研究结果相一致。同时钙处理可以抑制细胞壁降解酶活性,从而减缓细胞壁组分的降解。本试验中,不同钙处理均显著抑制了 PME、PG、CX 酶活性,且这 3 种酶都与果实硬度之间存在着显著相关性。说明细胞壁降解酶在果实软化中起着重要的作用。这与罗自生^[23]研究结果一致,同时欧志峰^[24]对‘红富士’苹果进行浸钙处理,研究发现钙处理可显著降低‘红富士’果实硬度下降程度,降低果实 PG 和 CX 活性,提高果实贮藏性。这与本试验研究结果相一致。王玲利等^[25]发现 PME 与细胞壁组分及果实硬度的相关性均不显著,并且钙处理并没有显著降低 PME 活性,认为 PME 在‘黄冠’梨果实衰老软化中的作用不明显。刘剑锋等^[26]也认为 PME 与果实硬度的下降关系不明显。

外源钙处理均能抑制果实细胞壁降解酶活性,抑制果胶、纤维素的降解,延缓果实硬度下降。但不同形态的钙处理之间也存在着差异。本试验研究表明,在硝酸钙、氯化钙、氨基酸钙、糖醇钙4种钙处理中,以糖醇钙的效果最为显著。糖醇钙处理能更好地保持果实硬度,且对果实可溶性果胶的上升、原果胶和纤维素的下降抑制作用更明显,同时能更好地抑制果实PME、PG、CX酶活性的上升。丁双双等^[27]和沈欣等^[28]研究也发现施用糖醇钙螯合形态的钙制剂,与硝酸钙、氯化钙等相比,能更好地提高的果实品质及产量。这可能是由于糖醇类物质能更好地螯合钙离子,使钙离子在果实内的移动性增强,从而促进果实对钙的吸收,提高钙养分的有效性,其内在机制有待进一步研究。

4 结 论

采后钙处理可有效降低‘寒富’苹果果实PME、PG、CX酶活性,抑制原果胶和纤维素的降解以及可溶性果胶的上升,从而较好地维持果实硬度。各处理中以糖醇钙处理效果最好。因此,建议采后使用2%糖醇钙浸泡果实15 min,可以显著提高果实硬度,延长苹果货架期。

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